Supplementary Information Guide for

Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress

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Supplementary Methods:

Chemical Standards and Solvent Materials

Mixtures of 16 PAHs (EPA M-610), PCBs (C-WNN) and PBDEs (BDE-CR) were purchased from AccuStandard (New Haven, CT, USA). Internal standards (Napthalene-d8, acenapthene-d10, phenathrene-d10, chrsyene-d12 and perylene-d12) and recovery standards (1-1'-biphenyl 2-fluroene-d10 and p-tertphenyl-d14) for PAH analysis were purchased from AccuStandard as mixtures (Z-014J-0.5X and CLP-LC-SS1). Internal standards PCB65, 155, and 204 for PCB analysis were individually purchased from AccuStandard. The recovery standard $^{13}C_{12}$-CB169 for PCB analysis was purchased from Wellington Laboratories (Guelph, Canada). The internal standard 4'-fluoro-2,3',4,6-tetrabromodiphenyl ether and recovery standard 4'-fluoro-2,3,3',4,5,6,-hexabromodiphenyl ether for PBDE analysis were both purchased from AccuStandard. All solvents and reagents, equal or above pesticide grade, were purchased from Fisher Scientific (Fisher Scientific, Fair Lawn, NJ, USA).

Sample Preparation

Water samples for each treatment were extracted for contents of PAHs, PCBs and PBDEs. Approximately 500 mL of water from the three marine plastic tanks were sampled and filtered through Whatman™ glass fiber filter paper (0.45 μm; GE Healthcare, Maidstone, Kent, UK). Filtered water samples were extracted by SPE using OASIS® glass cartridges (Waters Corporation, Milford, MA, USA) conditioned with DCM followed by acetone followed by milli-Q water. Following extraction, cartridges were spiked with known amounts of each internal

standard and eluted with acetone followed by DCM. Extracts were concentrated under nitrogen flow and resuspended in hexane. Final extracts were spiked with known amounts of recovery standards and analyzed by GC/MS.

Samples of ground LDPE samples were extracted for contents of 12 PAHs, 27 PCBs and 13 PBDEs (Figure 1). Approximately one gram of ground marine- and virgin-LDPE were extracted twice by sonication in 30 mL dichloromethane (DCM) and concentrated under nitrogen flow. For clean-up, we used solid phase extraction (SPE) with silica based cartridges (Waters Corporation, Milford, MA, USA) eluted with 100% hexane and 4:1 DCM/hexane. Final extracts were spiked with known amounts of recovery standard prior to GC/MS analysis.

Diet samples for each treatment were extracted for contents of 6 PAHs, 10 PCBs and 7 PBDEs (Figure 1). Approximately seven grams of sample were extracted twice by sonication in 30 mL 50:50 acetone/hexane and concentrated under nitrogen flow. Clean-up steps consisted of gel permeation chromatography (GPC; J2 Scientific, Columbia, MO, USA) to remove the lipids followed by SPE with silica-based cartridges. For GPC, samples were resuspended in 5 mL 1:1 ethyl acetate/cyclohexane. The method was optimized to ensure recovery of target analytes. The GPC column has a 2 cm i.d. and a length of 22.5 cm and is packed with 24 g of BioBeasd S-X3 in 1:1 ethyl acetate/cyclohexane. The flow rate was 5 mL/minute and the mobile phase was 1:1 ethyl acetate/cyclohexane. The eluent fraction between 10 and 22 minutes was collected and concentrated to 2 mL under a flow of nitrogen gas for SPE. For clean-up by SPE we followed the same methods as above for samples of LDPE. Final extracts were spiked with known amounts of recovery standard prior to GC/MS analysis.

After one month of exposure 12 fish were sampled for contents of 6 PAHs, 10 PCBs and 7 PBDEs (Figure 1). After two months, all fish remaining at the end of the exposure, excluding

those sampled for histopathology and gene expression endpoints, were sampled for body burden analysis. Prior to body burden analysis, fish were dissected and the stomach, liver and gall bladder were surgically removed. Remaining fish tissue samples were grouped into approximately 5 g composite samples and homogenized by mortar and pestle. One composite sample per replicate tank was analyzed after one month of exposure, and three composite samples per replicate tank were analyzed after two months of exposure. The extraction and clean-up procedures were the same as the diet samples. Extractable lipid content was determined gravimetrically using 10% of the sample extract prior to clean-up by GPC. Concentraitons of PAHs, PCBs and PBDEs are reported as ng/g lipid.

Chemical Analyses

Sample extracts for PAHs and PCBs in all sample matrices and PBDEs in water and fish tissue were analyzed using an Agilent 6890 series gas chromatograph and Agilent 5973 mass spectrometer (Santa Clara, CA, USA) with ultrapure grade helium (99.995%: Airgas West El Cajon, CA, USA) as the carrier gas and a Restek Rxi-5 Sil MS column (30 m × 0.25 mm i.d. × 0.25 µm thickness) integrated with a 5 m guard column. Selected ion monitoring (SIM) was used to detect 16 PAHs, 27 indicator PCBs, 12 PBDEs and internal and recovery standards. Instrumental parameters used in this study for PAHs and PCBs for all matrices and PBDEs in fish tissue were developed previously in our lab[59]. Ground LDPE and PC diet sample extracts were analyzed for PBDEs at the Southern California Coastal Water Research Project (SCCWRP; Costa Mesa, CA) using an Agilent 7890 gas chromatograph coupled to a 5975C quadrupole mass selective detector (Wilmington, DE, USA) with ultrahigh purity (>99.999%) helium as the carrier gas with a constant flow rate of 1.9 ml/min and an Agilent Agilent J&W DB-XLB column (30 m × 0.25 mm × 0.25 µm column). One microliter sample was injected at 300°C in

splitless mode. The oven temperature was programmed from 90°C held for 1 min to 150°C at 5°C/min, to 260 °C at 3°C/min, and to 320°C at 20°C/min held for 5 min. MS conditions are as follows: negative chemical ionization (NCI) with an ion source and quadrupole temperature of 150°C. The MSD was operated under the selected ion monitoring (SIM) mode to detect 12 PBDEs and internal and recovery standards.

Quality Assurance and Quality Control

Glassware was cleaned and muffled at 450˚C for 6 h. Surgical tools were solvent rinsed in acetone and hexane and glass fiber filter papers were muffled at 450˚C for 6 h. During extraction, samples were covered with aluminum foil to prevent contamination. Laboratory procedural blanks (virgin plastics for plastic samples and sodium sulfate for diet and tissue samples) and a spiked matrix blank (virgin pellets for plastic samples, sodium sulfate for diet samples and anchovies for tissue samples) were extracted and run with every sequence of 6 samples. Three quality control criteria were used to guarantee correct identification of target compounds: GC retention times matched those of standard compounds within ± 0.2 minutes, signal-to-noise ratio was greater than five, and the ratio between the quantitation and confirmation ions of each target compound was within ± 25% of the theoretical value.

The recovery of the internal standards had means ranging from 59-95% (n = 2) for ground LDPE, 51-75% (n = 3) for treatment diets, and 58-136% (n = 36) for fish tissue. The reported concentrations of total PAHs, PCBs and PBDEs are recovery corrected based upon the recovery efficiencies of internal standards. In spiked matrix blank samples, recoveries for the 16 PAHs were > 58% for ground LDPE, > 90% for PC diet, and > 58% for the 6 PAHs measured in fish tissue. Recoveries for the spiked matrix blank samples for the 27 PCBs were > 78% for ground LDPE, > 70% for PC diet, > 64% for the 10 PCBs measured in fish tissue. Finally,

recoveries for the matrix spiked blank samples for the 12 PBDEs were > 85% for ground LDPE, > 49% for PC diet, > 96% for the 7 PBDEs measured in fish tissue. Blank levels of the 16 PCBs, 27 PCBs and 12 PBDEs measured in laboratory procedural blanks were subtracted from the reported concentrations of total PAHs, PCBs and PBDEs extracted from samples.

References

59. Van, A., Rochman, C. M., Flores, E. M., Hill, K. L., Vargas, E., Vargas, S. A., Hoh, E. Persistent organic pollutants in plastic marine debris found on beaches in San Diego, California. *Chemosphere*. **3**, 258-263 (2012).

Supplementary Tables:

Supplementary Table S1. Concentrations of all PAH, PCB and PBDE congeners measured in LDPE (ng/g pellet), Treatment Diets (ng/g), and medaka after the 1- and 2-month dietary exposure in (ng/g lipid ± s.e.m.; n=3). Tissue samples are provided as average concentrations in ng/g lipid ± SE for PAHs (top), PCBs (middle) and PBDEs (bottom). Individual congeners are listed along the top row and treatments are listed down the first column. 2-factor ANOVAs showed significant differences between treatments for chrysene ($P = 0.006$), PCB 28 ($P = 0.022$) and PBDE congeners 47 ($P = 0.001$), 49 ($P = 0.003$), 99 ($P = 0.004$), 100 ($P = 0.002$), 153 ($P = 0.006$) and 154 ($P = 0.04$). Post-hoc SNK tests distinguished the marine-plastic treatment having greater concentrations of each of the above congeners from the virgin-plastic and control Treatments.

PAHs

Matrix	phenanthrene	anthracene	fluoranthene	pyrene	benzo(a)anthracene	chrysene
virgin LDPE (ng/g pellet)	25	4.8	2.4	nd	nd	nd
marine LDPE (ng/g pellet)	10	1.4	68	37	10	2.5
control diet (ng/g)	11	12	0.6	0.5	nd	nd
virgin-plastic diet (ng/g)	25	31	2.0	nd	nd	nd
marine-plastic diet (ng/g)	23	29	2.0	1.4	nd	nd
control fish (1 mo, ng/g lipid)	1.6 ± 0.3	nd	0.2 ± 0.0	nd	1.7 ± 0.9	2.6 ± 1.4
virgin-plastic fish (1 mo, ng/g lipid)	1.5 ± 0.3	0.1 ± 0.1	0.3 ± 0.2	nd	2.9 ± 1.5	2.7 ± 1.6
marine-plastic fish (1 mo, ng/g lipid)	1.7 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	nd	0.2 ± 0.2	2.0 ± 1.4
control fish (1 mo, ng/g lipid)	16 ± 1.9	0. 7 ± 0.4	2.7 ± 0.4	0.3 ± 0.3	49 ± 14	6.3 ± 1.4
virgin-plastic fish (2 mo, ng/g lipid)	26 ± 12	0.0 ± 0.0	6.9 ± 2.9	0.0 ± 0.0	87 ± 18	4.1 ± 1.8
marine-plastic fish (2 mo, ng/g lipid)	15 ± 2.6	20 ± 14	4.1 ± 1.4	0.0 ± 0.0	126 ± 34	14 ± 1.9*

PCBs

Matrix	PCB18	PCB28	PCB52	PCB44	PCB101	PCB123	PCB118	PCB153	PCB138	PCB187
virgin LDPE (ng/g pellet)	nd	nd	0.3	nd	0.5	0.3	nd	nd	nd	nd
marine LDPE (ng/g pellet)	0.5	0.3	2.1	1.5	2.9	1.2	2.7	2.5	2.5	0.6
control diet (ng/g)	0.1	nd	nd	nd	nd	nd	nd	0.1	nd	nd
virgin-plastic diet (ng/g)	0.1	nd	0.1	nd	0.1	nd	0.2	nd	0.2	0.1
marine-plastic diet (ng/g)	0.1	nd	0.1	nd	0.1	4.3	0.2	0.2	0.2	0.1
control fish (1 mo, ng/g lipid)	0.1 ± 0.0	1.4 ± 0.1	9.0 ± 1.0	3.1 ± 0.4	7.8 ± 0.9	0.8 ± 0.4	11 ± 1.0	8.4 ± 1.0	8.1 ± 0.9	0.6 ± 0.1
virgin-plastic fish (1 mo, ng/g lipid)	0.1 ± 0.0	1.5 ± 0.1	9.6 ± 0.5	3.2 ± 0.2	7.6 ± 0.6	nd	9.8 ± 0.1	6.6 ± 0.4	6.1 ± 0.3	0.4 ± 0.0
marine-plastic fish (1 mo, ng/g lipid)	0.1 ± 0.0	1.6 ± 0.1	8.8 ± 0.9	3.1 ± 0.2	7.3 ± 0.7	0.4 ± 0.4	12 ± 2.3	6.9 ± 1.4	6.5 ± 1.7	0.5 ± 0.1
control fish (1 mo, ng/g lipid)	0.5 ± 0.2	9.7 ± 0.4	74 ± 4.9	25 ± 1.8	78 ± 6.2	8.5 ± 1.8	107 ± 7.8	81 ± 7.0	78 ± 7.5	5.9 ± 0.5
virgin-plastic fish (2 mo, ng/g lipid)	0.6 ± 0.2	6.2 ± 1.6	71 ± 4.6	23 ± 1.6	83 ± 5.9	5.4 ± 1.7	96 ± 5.8	71 ± 5.3	65 ± 5.2	6.2 ± 0.6
marine-plastic fish (2 mo, ng/g lipid)	0.7 ± 0.2	11 ± 1.3*	89 ± 9.9	30 ± 3.1	101 ± 11	10 ± 2.3	126 ± 15	95 ± 10	88 ± 9.4	7.4 ± 0.8

PBDEs

Matrix	BDE49	BDE47	BDE100	BDE99	BDE155	BDE154	BDE153
virgin LDPE (ng/g pellet)	0.017	0.73	0.16	0.28	0.03	0.07	nd
marine LDPE (ng/g pellet)	0.10	0.54	0.25	0.40	0.02	0.07	0.46
control diet (ng/g)	0.01	0.14	0.05	0.10	0.02	0.02	0.72
virgin-plastic diet (ng/g)	0.03	0.24	0.09	0.16	0.09	0.03	1.76
marine-plastic diet (ng/g)	0.03	0.21	0.10	0.15	0.18	0.05	2.40
control fish (1 mo, ng/g lipid)	nd	56 ± 5.8	5.5 ± 1.5	2.9 ± 1.8	2.2 ± 1.1	4.3 ± 1.1	2.6 ± 1.4
virgin-plastic fish (1 mo, ng/g lipid)	nd	63 ± 3.3	3.4 ± 0.7	6.3 ± 3.1	2.9 ± 1.4	3.4 ± 0.5	3.5 ± 1.8
marine-plastic fish (1 mo, ng/g lipid)	nd	65 ± 14	4.8 ± 2.6	7.5 ± 1.6	3.9 ± 0.4	4.0 ± 1.6	2.0 ± 2.0
control fish (1 mo, ng/g lipid)	69 ± 7.1	58 ± 3.8	7.0 ± 0.4	4.4 ± 1.0	3.3 ± 0.2	5.0 ± 0.4	3.7 ± 0.4
virgin-plastic fish (2 mo, ng/g lipid)	82 ± 5.0	67 ± 5.3	8.6 ± 0.6	7.6 ± 1.5	1.9 ± 0.8	4.4 ± 1.1	2.5 ± 1.0
marine-plastic fish (2 mo, ng/g lipid)	122 ± 15*	109 ± 13*	11 ± 0.9*	11 ± 1.5*	2.7 ± 0.9	7.5 ± 0.9*	6.3 ± 0.7*

Supplementary Table S2. 2-factor ANOVA tables for Total PAHs, PCBs and PBDEs in fish at the 2-month sampling period. For each Cochran's test, ns denotes non-significance. SNK results are given in order from greatest to least concentration for each contaminant group among treatments. Where Tank(Treatment) was not significant at ≥0.25, $MS_{Tank(Treatment)}$ is pooled with $MS_{Treatment}$.

2 month PAHs
2-way ANOVA

		anthracene		benzo(a)anthracene		chrysene		fluoranthene		phenanthrene		pyrene		Total PAHs	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	1104.9	0.99(0.42)	13124	1.58(0.28)	167.45	0.06(0.01)	41.002	1.34(0.28)	303.18	0.60.83)	0.2542	1.00 (0.38)	23558	1.87(0.23)
Tank(Tr)	6	1113.2	2.41(0.07)	8313.5	2.17(0.10)	27.356	pooled	16.299	pooled	544.90	pooled	0.2542	pooled	12608	3.18(0.03)
Residual	18	462.45		3836.9		25.281		36.377		443.19		0.2542		3961.3	
Cochran's test		C=0.9984		C=0.400 ns		C=0.2958 ns		C=0.4570 ns		C=0.9560**		C=1.0000**		C=0.4310 ns	
SNK						OP>C=VP								MP(1>2=3)	

2 month PCBs
2-way ANOVA

		PCB 18		PCB 28		PCB 44		PCB 52		PCB 101		PCB 118		PCB 123		PCB 138		PCB 153	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	0.0718	0.18(0.83)	58.6844	4.53(0.02)	94.050	1.52(0.29)	856.75	2.00(0.16)	1259.6	2.06(0.15)	2004.8	2.12(0.14)	52.589	1.49(0.25)	1150.7	1.56(0.28)	1212.0	2.26(0.13)
Tank(Tr)	6	0.4276	pooled	6.0311	pooled	61.696	1.55(0.22)	502.23	pooled	480.26	pooled	758.79	pooled	4.4522	pooled	737.38	1.69(0.18)	529.97	pooled
Residual	18	0.3781		15.274		39.771		403.33		655.96		1008.6		45.682		435.04		536.89	
Cochran's test		C=0.2415 ns		C=0.2598 ns		C=0.4447 ns		C=0.4868 ns		C=0.4132 ns		C=0.5417 ns		C=0.2518 ns		C=0.3826 ns		C=0.3516 ns	
SNK				MP=C>VP															

		PCB 187		Total PCBs	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	5.8829	1.61(0.22)	38821	2.34(0.12)
Tank(Tr)	6	2.8224	pooled	16043	pooled
Residual	18	3.9198		16730	
Cochran's test		C=0.3273 ns		C=0.4250 ns	
SNK					

2 month PBDEs
2-way ANOVA

		BDE 47		BDE 49		BDE 99		BDE 100		BDE 153		BDE 154		BDE 155		Total BDEs	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	6668.7	10.22(0.001)	6837.3	7.60(0.003)	112.09	7.02(0.004)	35.787	8.34(0.002)	32.634	6.30(0.006)	24.623	3.66(0.04)	4.0243	0.89(0.43)	35948	11.49(0.0003)
Tank(Tr)	6	242.76	pooled	236.72	pooled	7.9341	pooled	1.2093	pooled	2.6093	pooled	3.7676	pooled	2.9983	pooled	544.60	pooled
Residual	18	788.88		1120.7		18.656		5.3195		6.0379		7.7098		5.0572		3990.5	
Cochran's test		C=0.6483		C=0.3681 ns		C=0.2061 ns		C=0.3832 ns		C=0.3646 ns		C=0.3326 ns		C=0.3032 ns		C=0.6106 ns	
SNK		MP>VP=C		MP>VP=C		MP=VP=C		MP>VP=C		MP>VP=C		MP>VP=C				MP>VP=C	

Supplementary Table S3. 1-factor ANOVA tables for Total PAHs, PCBs and PBDEs in fish at the 1-month sampling period. For each Cochran's test, ns denotes non-significance. SNK results are given in order from greatest to least concentration for each contaminant group among polymers and locations.

1 month PAHs
1-way ANOVA

		anthracene		benzo(a)anthracene		chrysene		fluoranthene		phenanthrene		pyrene		Total PAHs	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	0.0117	1.45(0.31)	5.6009	1.74(0.25)	0.3683	0.06(0.95)	0.0114	0.27(0.77)	0.0443	0.019(0.83)	N/A	N/A	7.8389	0.71(0.53)
Residual	6	0.0081		3.2166		6.6981		0.0415		0.2275		N/A		10.9998	
Cochran's test		C=0.5165 ns		C=0.7279 ns		C=0.4004 ns		C=0.5422 ns		C=0.4514 ns		N/A		C=0.5286 ns	

1 month PCBs
1-way ANOVA

		PCB 18		PCB 28		PCB 44		PCB 52		PCB 101		PCB 118		PCB 123		PCB 138		PCB 153	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	0.0001	0.56(0.60)	0.0314	2.88(0.13)	0.0346	0.14(0.87)	0.5584	0.28(0.77)	0.2075	0.13(0.88)	3.5235	0.55(0.14)	0.4378	1.50(0.30)	3.0998	0.82(0.49)	2.8145	0.90(0.46)
Residual	6	0.0003		0.0109		0.2405		2.0006		1.6057		6.4364		0.2913		3.7910		3.1423	
Cochran's test		C=0.5650 ns		C=0.4231 ns		C=0.5179 ns		C=0.5040 ns		C=0.4625 ns		C=0.8409 ns		C=0.5296 ns		C=0.7467 ns		C=0.6557 ns	

		PCB 187		Total PCBs	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	0.0285	1.00(0.42)	19.611	0.28(0.76)
Residual	6	0.0286		68.914	
Cochran's test		C=0.5012 ns		C=0.5982 ns	

1 month PBDEs
1-way ANOVA

		BDE 47		BDE 49		BDE 99		BDE 100		BDE 153		BDE 154		BDE 155		Total BDEs	
Source	**df**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(0.84)**	**MS**	**F(P)**	**MS**	**F(P)**	**MS**	**F(P)**
Treatment	2	59.896	0.25(0.79)	N/A	N/A	17.004	1.13(0.38)	3.5866	0.39(0.70)	1.7314	0.18	0.7266	0.18(0.84)	2.2041	0.63(0.56)	128.96	0.18(0.84)
Residual	6	242.62		N/A		15.072		9.3149		9.3831		4.0311		3.4844		698.31	
Cochran's test		C=0.8186 ns		N/A		C=0.6208 ns		C=0.7019 ns		C=0.4334 ns		C=0.6298 ns		C=0.6003 ns		C=0.6705 ns	

Supplementary Table S4. Primers used for quantitative polymerase chain reaction (qPCR)[56,57].

Primer Name	Accession Number	Forward Primer 5'-3'	Reverse Primer 5'-3'
CYP1A	AY297923	CGCAGAAAGTTGGCCTACAGT	TCTGCATTGCTGCCCTCTAG
GADPH	AV671008	TGTGGAAAAGGCCTCACTTCA	CAGACACGACCACACGCTGT
β Actin	S74868	TCCACCTTCCAGCAGATGTG	AGCATTTGCGGTGGACGAT
18SrRNA	AB105163	CGTTCAGCCACACGAGATTG	CCGGACATCTAAGGGCATCA